ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENTS WORKSHOP

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INTRODUCTION

The advent of new alternative methods (NAMs) for generating safety information on chemicals provides an opportune time to take stock of what chemical risk assessments could/should look like in the 21st century. This workshop gathered international regulatory agencies and their science support colleagues to discuss progress in applying the new tools to prioritization, screening, and quantitative risk assessment of differing levels of complexity. To date, most progress in applying NAMs has been in screening and prioritization, but ultimately to modernize quantitative risk assessment, there is a need to demonstrate how the data and tools can be incorporated into future risk assessments, in particular for data poor chemicals. Scientific and regulatory needs for the quantitative application of NAMs to risk assessments were identified, and example case studies designed to address them were explored. Case study proposals are being drafted, and will ultimately be submitted to or conducted by multinational groups, including OECD (Organization for Economic Co-operation and Development), following the workshop.

Presentation abstracts were written by the presenters. US EPA's contractor, ICF, summarized the question and answer (Q&A) sessions and the facilitated discussions.

The agenda is provided in Appendix A, and the list of participants is provided in Appendix B.

DAY 1 – WEDNESDAY, SEPTEMBER 14, 2016

Welcome

Jim Jones, US Environmental Protection Agency, USA

Mr. Jim Jones, Assistant Administrator of the Office of Chemical Safety and Pollution Prevention (OCSPP) at the United States Environmental Protection Agency (US EPA), thanked Dr. Robert Kavlock for his leadership, and for leading the agency and the world in using new alternative methods (NAMs) to inform risk assessment. He explained that OCSPP regulates pesticides and industrial commercial chemicals, and does more risk assessments than any other US agency. They have hundreds of chemicals in their jurisdiction, ranging from data-rich to data-poor, enabling them to be a proving ground for new evolving technologies. Over the years OCSPP has tried to advance the application of these technologies (for example, ToxCast, RapidTox, etc.) by using them in a practical way to inform risk assessment. The only way to successfully handle the extraordinary large number of chemicals in the US and the rest of the world will be to utilize emerging technology.

Thomas A. Burke, US Environmental Protection Agency, USA

Dr. Thomas Burke, Deputy Assistant Administrator of the Office of Research and Development (ORD) at US EPA, explained the aim of the meeting and insisted it be a turning point in chemical risk assessment. The people, leadership, commitment, and goals are all in place. He reminded the group that US EPA's core mission is to protect public health, and the Agency's ability to accomplish that mission depends on the application of science. It is evident that the old methods and timeframes do not work for the current risk contexts. Without sufficient information, US EPA does not (and cannot) take regulatory action, which is why these new methods are so important. He emphasized the critical need to set a course for collaborating on the application of these new methods during the meeting in order to define the future of chemical risk assessments.

Robert J. Kavlock, US Environmental Protection Agency, USA

Dr. Robert Kavlock, Deputy Assistant Administrator of ORD at US EPA, emphasized that chemicals do not know geographic borders, which is what brings the workshop participants together. He stated his belief that significant benefits will be realized by working together to accelerate the pace of risk assessment.

The NICNAS Inventory Multi-tiered Assessment and Prioritisation (IMAP) Program

Kerry Nugent, National Industrial Chemicals Notification and Assessment Scheme, Australia

The Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework was established by the Australian Government's National Industrial Chemicals Notification and Assessment Scheme (NICNAS) to accelerate the assessment of risks posed to human health and the environment by previously unassessed chemicals. The objectives of IMAP were the identification and rapid assessment of existing chemicals of concern, leading to enhancements in chemical safety information flow and chemicals management.

IMAP comprises three tiers of assessment, with the assessment effort increasing with each tier. The initial two tiers combine assessment and prioritisation. Tier I utilises a matrix-based sorting step, which is focussed on identifying chemicals of sufficiently low regulatory concern as to not require further assessment or other use of resources. Tier II involves identification of relevant data, and preparation of a brief report to characterise the likely risks. The Tier II assessments also examine whether appropriate risk management measures already exist, and whether the available data are sufficient to justify

relevant risk management measures. Tier III comprises assessment of any critical questions identified in the Tier II examination of the available data.

For the majority of chemicals, IMAP assessments were undertaken in the absence of any Australian use or volume data, which limited the extent to which quantitative assessment could be carried out. In addition, only approximately 10 percent of Tier II assessments had data for all standard toxicological endpoints considered.

This presentation will focus on the human health aspects of IMAP, which also includes environmental assessment. The IMAP matrix used for human health at Tier I was developed to account for the lack of quantitative data, together with the need to consider a wide range of hazards. Unlike the Risk21 matrix, which uses effect levels and doses as its axes, the IMAP matrix used surrogates for these quantities, described as hazard bands and exposure bands.

At Tier II, the absence of access to detailed exposure information prevented the use of margin of safety approaches. However, risk management recommendations were able to be made based on qualitative risk assessment approaches for a significant number of chemicals.

The challenges of the use of non-standard data sources, including read-across, grouping and quantitative structure-activity relationships (QSAR), in as part of the IMAP framework will be discussed. The extent to which non-quantitative risk assessment can be used to inform risk management will also be addressed in the presentation.

DISCUSSION AND Q&A

- Dr. Kerry Nugent provided clarification on the product of Tier 2, which is a match between hazard and scenarios of use for a chemical. It is beyond hazard, but does not contain a quantitative component of risk.
- Dr. Warren Casey asked if pesticides and their ingredients were covered; Dr. Nugent replied they were not.
- Dr. Gina Solomon mentioned that Dr. Nugent's talk implied NICNAS used a threshold of concern approach and asked him to elaborate.
- There was discussion regarding the difference between US and Australian approaches to risk assessment, and how much emphasis each program places on producing a single quantitative value. Determining a specific value can be an onerous exercise, and while it has value, it may not be the thing most critical aspect for risk management.

New Approach Methodologies to Support Canada's Chemicals Management Plan

Tara Barton-Maclaren, Health Canada, Canada

Under the Chemicals Management Plan (CMP), the Government of Canada is committed to addressing 4,300 existing substances by 2020. Moving forward into the third phase of the CMP (2016–2020) and beyond, a key challenge is assessing the potential for risk to human health of substances that have limited to no toxicological data. The *Canadian Environmental Protection Act 1999* (CEPA) requires the incorporation of weight-of-evidence and precaution and that risk assessment conclusions are protective of human health and the environment. In addition, the assessment methods must be able to accommodate substances and substance groupings with varying amounts and types of information, including emerging scientific knowledge and assessment approaches. As such, Health Canada has an interest in establishing proof of concept for the application of new approach methodologies, including High Throughput Screening (HTS) data, into risk assessment activities under the CMP. Through active collaborations with the Environmental Health Science and Research Bureau at Health Canada, as well as

with international partners (e.g. US EPA, Organization for Economic Co-operation and Development (OECD)), progress has been made on the interpretation of emerging data and NAMs for a range of uses in risk assessment from priority setting to informing decision-making.

Canada's presentation will provide an overview of the various analyses that have been conducted or are currently in progress that explore the use of NAMs in order to gain confidence for broader application in risk assessment activities under CEPA. A particular focus will be on an ongoing joint Health Canada-US EPA National Centre for Computational Toxicology (NCCT) case study developed to gain experience using a subset of 21 substituted phenols that will be addressed under phase 3 of Canada's CMP. A human health related concern with phenols is that they can have the potential to be estrogenic. Bisphenol A (BPA) is a typical example of a phenolic estrogen. The selected CMP phenolic compounds contain substituents at various positions relative to the hydroxyl group. The type of substituent and position relative to the hydroxyl group is anticipated to have an impact on the estrogenic potential and potency. This case study addresses several key elements including investigating systematic approaches for identifying valid source analogues and assessing their resulting read-across performance as well as exploring the utility of HTS data to substantiate chemical categories formed and reducing uncertainties associated with the traditional read-across for apical effects. (Q)SARs such as those derived under the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) are also integrated into the weight of evidence assessment. Where the required data was available for target CMP substituted phenols, the bioactivity exposure ratio (BER) was compared with traditional margin of exposure (MOE) techniques in order to further examine the utility of the HTS data to predict potential level of concern for human health effects for the purposes of prioritisation and risk assessment.

The case study is still ongoing but work completed to date shows that the approach is promising for developing a weight of evidence assessment for the estrogenicity activity for the target CMP substituted phenols.

DISCUSSION AND Q&A

- Dr. John Bucher asked whether the phthalate assessment considered both parent compounds and monoesters or only monoesters. Dr. Barton-Maclaren responded high-throughput data for both parent compounds and monoesters exist, but interpretation of these data is complex. To simplify the interpretation of the data, the assessment considered monoesters only.
- A second participant asked if the group had reached any conclusions related to regulatory decision making. Dr. Barton-Maclaren replied that the group continues to explore the use of the BER approach in de-prioritizing substances with low exposure and no/low activity. They are also continuing to explore integrating NAMs into the Integrated Approaches to Testing and Assessment (IATA) context and showing the proof of concept in combination with more traditional data.
- Dr. John Vandenberg asked where the BER approach, especially for chemicals with small BERs, fits in from screening to full assessment. Dr. Barton-Maclaren replied that the group is using BERs to screen for chemicals on the low end of bioactivity and the low end of exposure. They do not plan to use BERs to draw conclusions for substances with higher activity or exposure, but the BER could identify those chemicals needing more information or requiring a more in-depth assessment.
- Dr. Maurice Whelan noted that the BERs are consistently lower than the MOEs and asked Dr. Barton-Maclaren to comment on why that may be. Related, he also asked how the group plans to validate the approach, for example by comparing the level of protection it offers in comparison to existing schemes. Dr. Barton-Maclaren stated that the group selected the assay

with the lowest activity, as opposed to the assay related to the pathway of toxicity, to generate conservative estimates. In terms of validation and acceptance, she replied that the group is looking to expand the study to include other international experiences. The goal of the presentation was to introduce the approach and discuss collaborations to expand and refine its application.

- Dr. Rusty Thomas noted the importance of discussing the meaning of negative responses, especially in the context of regulatory decision making. Dr. Barton-Maclaren responded that to classify a substance as low priority based on a negative assay response, the substance would also need to fall within the no/low exposure context.

REACH and CLP and the Use of New Approach Methods Information

Mike Rasenberg, European Chemical Agency, European Union

REACH is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals. The CLP Regulation ensures that the hazards presented by chemicals are clearly communicated to workers and consumers in the European Union through classification and labelling of chemicals.

Companies are responsible for collecting information on the properties and uses throughout the supply chain, of the substances they manufacture or import above one tonne a year. They also have to assess the hazards and potential risks presented by the substance. This information is communicated to ECHA through a **registration** dossier containing the hazard information and, where relevant, an assessment of the risks that the use of the substance may pose and how these risks should be controlled throughout the supply chain. All submitted dossiers are verified for 'completeness', but not for compliance.

Registration applies to substances on their own, substances in mixtures and certain cases of substances in articles. Chemical substances that are already regulated by other legislations such as medicines, or radioactive substances are partially or completely exempted from REACH requirements.

ECHA and the Member States **evaluate** some the information submitted by companies to examine the compliance of the registration dossiers and evaluate testing proposals and to clarify if a given substance constitutes a risk to human health or the environment. Evaluation under REACH focuses on three different areas:

- Examination of testing proposals submitted by registrants: no new animal test should be done, without a verified proposal first
- Compliance check of the dossiers submitted by registrants: to verify the compliance of at least 5% of dossiers per tonnage band
- Substance evaluation to clarify if there are risks to human health or the environment.

Once the evaluation is done, registrants may be required to submit further information on the substance.

The **authorisation** procedure aims to assure that the risks from Substances of Very High Concern are properly controlled and that these substances are progressively replaced by suitable alternatives while ensuring the good functioning of the EU internal market.

Substances with the following hazard properties may be identified as Substances of Very High Concern (SVHCs):

- Substances meeting the criteria for classification as carcinogenic, mutagenic or toxic for reproduction category 1A or 1B in accordance with Commission Regulation (EC) No 1272/2008 (CMR substances)
- Substances which are persistent, bioaccumulative, and toxic (PBT) or very persistent and very bioaccumulative (vPvB) according to REACH (Annex XIII)
- Substances identified on a case-by-case basis, for which there is scientific evidence of probable serious effects that cause an equivalent level of concern as with CMR or PBT/vPvB substances

After a two-step regulatory process, SVHCs may be included in the Authorisation List and become subject to authorisation. These substances cannot be placed on the market or used after a given date, unless an authorisation is granted for their specific use, or the use is exempted from authorisation.

Manufacturers, importers or downstream users of a substance on the Authorisation List can apply for authorisation.

Restrictions are a tool to protect human health and the environment from unacceptable risks posed by chemicals. Restrictions may limit or ban the manufacture, placing on the market or use of a substance. A restriction applies to any substance on its own, in a mixture or in an article, including those that do not require registration. It can also apply to imports.

The **CLP Regulation** ensures that the hazards presented by chemicals are clearly communicated to workers and consumers in the European Union through classification and labelling of chemicals. In most of the cases, suppliers need to decide on the classification of a substance or mixture (self-classification). Certain situations require that the classification of a substance is harmonised and made obligatory at Community level to ensure an adequate risk management throughout the European Community. Manufactures and importers need to notify the classification and labelling of substances and substances in mixtures.

Most of the information in the REACH and CLP dossiers is published on ECHA's website. For information on chemicals: https://echa.europa.eu/information-on-chemicals . The information is also made available via the OECD eChemPortal: http://www.echemportal.org/

Companies manufacturing and importing chemicals in the EU/EEA are responsible for the safe use of their products. As registrants, they need to assess if their chemicals may cause adverse effects to human health and the environment. This is done based on reliable test results or by alternative information which is scientifically justified. What reliable test results are, is stipulated in the Commission Regulation on test methods, REACH Article 13 and ECHA guidance. In general the standard requirements are studies conducted using/based on OECD Test Guidelines and EU Test Methods.

The **standard information requirements** are those which are required as a minimum to meet the registration obligations of REACH. They depend on the quantity of the substance that is manufactured or imported into the EU/EEA and are described in Annexes VI to X to REACH. These minimum data requirements may be adapted as appropriate. This means that certain tests may be waived. In general, **adaptations** can be done for three different reasons:

1. Testing is not scientifically necessary
2. Testing is not technically possible
3. Testing is not needed because of demonstrated low exposure

These general rules are detailed in REACH Annex XI. The specific rules and adaptation possibilities for each information requirement are detailed in column 2 of REACH Annexes VII to X. The purpose of the standard information requirement is (to contribute to) classification and/or risk assessment. Alternative

approaches should meet the same requirement; they should be fit for classification and/or risk assessment.

Testing does **not** appear **scientifically necessary** under the following items:

1. Use of existing data
2. Weight of evidence
3. Qualitative or Quantitative structure-activity relationship ((Q)SAR)
4. In vitro methods
5. Grouping of substances and read-across approach

For the discussion of the use of NAM data item 4 and 5 are of relevance.

The **in vitro methods** have requirements divided between positive and negative results.

For positive results:

- Results obtained from suitable *in vitro* methods may indicate the presence of a certain dangerous property or may be important in relation to a mechanistic understanding, which may be important for the assessment.
- In this context, 'suitable' means sufficiently well-developed according to internationally agreed test development criteria (e.g. the JRC's EURL ECVAM (EU Reference Laboratory for Alternatives to Animal Testing)criteria for the entry of a test into the pre-validation process).
- Depending on the potential risk, immediate confirmation requiring testing beyond the information foreseen in Annexes VII or VIII or proposed confirmation requiring testing beyond the information foreseen in Annexes IX or X for the respective tonnage level may be necessary.

For negative results

- If the results obtained from the use of such *in vitro* methods do not indicate a certain dangerous property, the relevant test shall nevertheless be carried out at the appropriate tonnage level to confirm the negative result, unless testing is not required in accordance with Annexes VII to X or the other rules in this Annex.
- Such confirmation may be waived, if the following conditions are met:
    1. results are derived from an *in vitro* method whose scientific validity has been established by a validation study, according to internationally agreed validation principles;
    2. results are adequate for the purpose of classification and labelling and/or risk assessment; and
    3. adequate and reliable documentation of the applied method is provided.

Grouping of substances and read-across is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under REACH. For grouping of substances and read-across approach the legal text stipulates that "Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances.

Under REACH, any read-across approach must be based on structural similarity between the source and target substances. However, structural similarity alone is not sufficient to justify the possibility to predict property(ies) of the target substance by read-across. A read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological property is possible and should be based on recognition of the structural aspects the chemical structures have in common and the differences between the structures of the source and target substances. The possibility for predictions of similar

properties should be linked to the common structural aspects. The differences in the chemical structures should not influence the toxicological properties or do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case. How read-across and categories are assessed under REACH is reflected in the Read-Across Assessment Framework (RAAF): https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/

Based on the above **NAM** could – at least in theory - be **used under REACH** on its own or in support of read-across for **definite hazard and risk assessment**. However, except in some specific situations, the data is so far used by Authorities to strengthen the evidence (e.g. endocrine properties), but not as 'stand-alone' information. To ECHA's current understanding, NAM has not been used for industry submissions under REACH (Registration dossiers).

ECHA and Member States **started to use NAM for prioritisation** of chemicals, specifically to find **indications of possible effects** and not to actively deprioritise. More specifically, it is taken into consideration for priority setting, if a substance has positive results to at least one of the in-vitro assays from ToxCast related to androgenic, estrogenic, and thyroid-related mode of action (MoA).

In general the main challenge is to understand the NAM data and its structures. For instance the use of standardised formats like OECD OHT 201 would be helpful. More critical is the challenge to understand the relevance of the data, in relation to relevant priority 'endpoints' and effects, which for REACH are indications for CMRs, PBTs, vPvBs, or a substance of equivalent concern (e.g., endocrine disruption).

In a **short term** the use of NAM (by regulatory authorities) could be increased and/or improved if there would be a better understanding of NAM in function of prioritisation and screening with a focus on endpoints related to the high priority areas of potential concern. In terms of specific endpoints, this would mean a focus on:

- Bioaccumulation
- Biodegradation
- Repeated dose toxicity
- Developmental toxicity
- Reproduction toxicity
- Carcinogenicity
- Genotoxicity
- Aquatic toxicity (long term)

In a **mid-term** future, within the current legal framework for chemicals management (like REACH), one could foresee the development of NAM to strengthen hypothesis for other alternative approaches (e.g. read-across), where 'current' OECD guidelines (based on effects in animals) act as reference. This approach would require a closer cooperation between regulatory authorities and research (authorities) and academia to bridge the gap between what is scientifically possible and what is legally required under the current framework.

In a **longer term** NAM might be fully integrated into approaches to manage risks of chemicals, offering a "better" prediction for possible impact of chemicals on man and/or the environment. This approach would also require a closer corporation between regulatory authorities and research (authorities). For this however, a reflection (revision?) on how hazard and risk assessment in the regulatory systems are defined and functioning today is needed.

DISCUSSION AND Q&A

- Dr. Stan Barone asked if the regulatory mandate treats new and existing chemicals differently. Mr. Mike Rasenberg replied that there is no longer a distinction between new and existing chemicals, and the requirements are the same for both. He clarified that the chemicals formally considered "new" are considered registered, as they have been fully scrutinized. He elaborated that "new" does not imply a new molecule, but a new substance for a given company. A company must register a substance at its first use, even if it has been registered by another company previously.
- Dr. Barone asked about differences between the European and US regulatory contexts. Mr. Rasenberg stated that in the US, new chemicals undergo some level of scrutiny and are subsequently approved, while in the EU it is a registration process, and substances are fine as long as the dossier is complete. The concept is to put the liability to companies via dissemination of information and verify compliance via compliance checks.
- Dr. Rusty Thomas commented that the requirement for the new approaches to be "as good or better" than traditional approaches is vague and asked for clarification on whether this refers to similar uncertainty and variability, false positives and false negatives, and error rates in predicting human toxicity. Mr. Rasenberg responded that, from a legal perspective, how well an animal study represents and predicts the human system is important, but it is not the prime consideration in what makes the evidence acceptable. Regulators have confidence in the current models, and changing the regulations will require building confidence in the new approaches.
- A participant asked, in terms of specific organ toxicity, if alternatives must be predictive of the category of specific organ toxicity or if they also must predict the new organ spectrum in that specific organ toxicity. Mr. Rasenberg responded that he would assume that the new approach would need to perform as well as the traditional approach.
- An attendee offered three comments. First, animal tests are the regulatory standard, so it is difficult to out-perform them. Second, variability is not incorporated often, but it would add valuable information. Finally, he noted that if experts believe that species-specific cell lines predict species-specific toxicity, then rat-based assays should be used to predict rat-based endpoints. He warned that from a legal perspective, approaches might not get adopted if human-specific assays do not match animal endpoints. Mr. Rasenberg admitted that he does not contest this, but was asked to provoke a critical discussion. The question of how to get to that point remains.
- Another attendee asked whether the regulations are similar for environmental toxicity. Mr. Rasenberg stated that ECHA has discussed environmental toxicity informally, but noted that these assessments are difficult, since the current approach relies heavily on extrapolation but building a better approach would be difficult.

Connecting Exposure, Toxicokinetics, and Toxicity: Towards Animal Free Risk Assessment in Food Safety?

Jean-Lou Dorne, European Food Safety Agency, European Union

Human risk assessment of chemicals in the food safety area involves the classic steps to bring hazard and exposure together for risk characterisation. Ideally, sound hazard identification and hazard characterisation requires a quantitative understanding of the mode of action, i.e., toxicokinetics (TK) and toxicodynamic (TD) processes in humans for compounds entering the human body via the oral route. A key issue is to move away from empirical approaches using test species and move towards human

relevant mechanistic approaches. This presentation explores some research and collaborative efforts at the European Food Safety Authority (EFSA) aiming to move towards mechanistic alternatives to animal testing in the food safety area through the developments of data-based and biologically-driven tools.

EFSA has published over 2000 risk assessments for over 4000 substances in the human health, animal health and the ecological areas since its creation in 2002. The development of "Openfoodtox": EFSA's open source database (12/2016), structured using OECD harmonised templates, and the nature of the summary hazard data available for individual substances is briefly discussed.

Since pesticides are of high concern and data rich chemicals, they could be used for testing advantages and limitations of new tools. In this context, EFSA ongoing and planned activities are presented including:

1) A database of validated endpoints for risk assessment covering human health and the environment
2) Use of non-animal strategies for addressing metabolites in the new EFSA guidance to define pesticides residues (chemicals to be included in the assessment of consumer risks)
3) 3AOPs (adverse outcome pathways) and identification of risks of human diseases not sufficiently covered by animal experimental studies
4) Realistic environmental risk assessments addressing landscape and spatial variability.

Collaborative research activities to develop an open source TK platform are presented with a focus on the basic principles to develop tools and models for human risk assessment. These include data collection on human variability in absorption, distribution, metabolism, excretion processes and the use of human *in vitro* and *in silico* tools to support the application of quantitative *in vitro* to *in vivo* extrapolation in food safety.

Ultimately, supporting risk assessors and decision makers requires an understanding of their practical needs (i.e., problem formulation, knowledge available for a chemical, resources and time available). Some examples of generic scenarios (data poor, regulated compound, data rich compound) and options to map and weigh evidence for exposure, hazard (TK/TD) and risk characterisation in the food safety area are illustrated. International cooperation between scientific advisory bodies throughout the world concludes as the cornerstone to (1) translate 21st century toxicological research into real life case studies, harmonized methodologies, and tools and (2) train the current and next generation of risk assessors.

DISCUSSION AND Q&A

- Dr. Stan Barone noticed Mr. Jean-Lou Dorne used zebrafish and trout in his TK work and asked if Mr. Dorne was looking at any other species that are commonly used for risk testing. Mr. Dorne noted that he had removed the ecotoxicology aspect from this talk, but he has used minnows, zebrafish, and trout in the TK work.
- Dr. Jose Tarazona briefly discussed the pesticide work conducted at EFSA, and then answered questions from the audience.
- Dr. Warren Casey stated that the US has a list of tests that must be performed before a new pesticide active ingredient can be registered and asked if EFSA has a similar standardized list. Dr. Tarazona explained that companies must submit a full dossier to EFSA, which has similar information requirements as in the US. In addition, EFSA requires the applicant to perform a literature search covering all active metabolites and chemicals, which provides them with lots of information and new methodologies used on the substance. EFSA then conducts a full assessment of the chemical, even if it has already been registered in another country.

- Dr. Robert Kavlock asked Dr. Tarazona if he has concerns about pesticide ingredients regulated by EFSA. Dr. Tarazona responded that he does, as EFSA only reviewed the active ingredients in pesticides in the past, and any chemical registered under REACH is permitted in pesticide formulations until it is banned. Discussions of risk assessments for these ingredients has begun and EFSA expects to perform the assessments.
- Dr. Tina Bahadori noted that US EPA regions identified bovine exposures to perfluorinated compounds as a pathway of interest and asked if Mr. Dorne has TK data that might inform that pathway. Mr. Dorne replied that EFSA has pig data for several congeners, but he was not sure about ruminant data.

Computational Risk Assessment for Mixtures of Chemicals: The Case of Aromatase Inhibitors

Phillippe Hubert, INERIS, France

Within the framework of EUROMIX project, the size of potential effects of random mixtures of aromatase inhibitors on the dynamics of women's menstrual cycle has been quantified through mathematical modeling and simulations. Combining computational toxicology with ExpoCast exposure data and ToxCast assay data gives access to predictions of human health risks stemming from realistic exposures to chemical mixtures. Random exposures were simulated to millions of potential mixtures of up to 256 aromatase inhibitors. A pharmacokinetic model of intake and disposition of the chemicals predicted their internal concentration as a function of time (up to two years). A ToxCast aromatase assay provided concentration-inhibition relationships for each chemical. The resulting total aromatase inhibition was input to a mathematical model of the hormonal hypothalamus-pituitary-ovarian control of ovulation in women. Above 10 percent inhibition of estradiol synthesis by aromatase inhibitors, noticeable (eventually reversible) effects on ovulation were predicted. Exposures to individual chemicals never led to such effects. Typically, more than 10 percent of the combined exposures simulated had mild to catastrophic impacts on ovulation. The size of the effects predicted is consistent with an increased risk of infertility in women from daily life exposures to our chemical environment.

The results demonstrate the possibility to predict large scale mixture effects for endocrine disrupters with a predictive toxicology approach. It is suitable for high throughput ranking and risk assessment, and it illustrates benefits and limitations of an approach for using data bases and pharmacokinetic (PK) modeling.

DISCUSSION AND Q&A

- Dr. Robert Kavlock noted that US EPA aims to make data publically available and pointed to Mr. Phillippe Hubert's work as an example of another organization using this type of data in an unexpected and beneficial way.
- Dr. Stan Barone asked for clarification on the modelled exposure and use-scenarios. Mr. Hubert explained that he obtained the exposures from ToxCast in milligrams per kilogram and averaged over two years. He added that the exposure work was mostly done by his INERIS colleague, Frederic Dubois.
- One participant noted that chemical mixtures are not random. Biomonitoring studies can be mined to determine chemicals that co-occur, which can then inform the exposure models. The participant asked if biomonitoring and co-occurrence data are available in Europe and, if so, whether INERIS has used it to model formulations of chemical mixtures. Mr. Hubert responded that those data are available and INERIS has utilized them. He also explained that INERIS has recognized the need to account for autocorrelation. He noted that considering the individual behaviors of chemicals is another method for assessing mixtures, but it is a complex method. He

added that the third approach needs more investment, as metabolite measurements or PK models are required to examine bioavailability.

- Dr. Jennifer Orme-Zavaleta agreed that there is a need for a global strategy on data collection and tool development. She explained that US EPA has been working to coordinate an approach across federal agencies and the International Society of Exposure Science planned to meet the following month to initiate a European strategy for exposure information. She noted that data collection and sharing is a significant challenge and asked how data access and collaborative tool development could be improved. Mr. Hubert recognized that data collection, sharing, and use is a challenge, but pointed to international workshops as a way to increase familiarity with other agency's resources and to build trust, as many countries working separately and still others may not have the tools or resources to generate that data.
- One participant pointed to an effort to capture newly generated toxicity data in a consistent manner. He noted that the data collection process was successful, and the next step will be to use the data to inform exposure and use. Mr. Hubert agreed that data construction is an important consideration for data sharing.

List of Chemicals in the Ministry of the Environment, Japan Progress of EXTEND2010. Results of Tier-1 Screening Assays for Candidate Substances, Aspect and Issues for Tier-1 Assessment and Tier-2 Testing

Taisen Iguchi, Yokohama City University, Japan

Ministry of the Environment, Japan, implemented their third program on endocrine disruption titled "EXTEND2010" (EXTEND: Extended Tasks on Endocrine Disruption) in July 2010.

Assessment framework of ecotoxicological effects has been developed, based on test protocols of fish (Medaka, *Oryzias latipes*), amphibian (*Xenopus laevis*) and invertebrates (*Daphania magna*) which have been developed through collaborations with UK, USA, and OECD. *In vitro* assays using receptors of fish (estrogen receptor, androgen receptor), amphibians (thyroid hormone receptor) and invertebrates (ecdysone receptor, juvenile hormone receptor) have also been developed in the ministry's program. Two-tiered framework for assessing endocrine disrupting effects of chemicals on organisms in the environment has developed. This framework is designed to effectively identify potential candidates for endocrine disruptors using available information and test results, not to examine all of the possible candidates in detail. The chemicals detected in the aquatic environment in the national monitoring programs have been nominated for the testing and assessment under the EXTEND2010. Reliability evaluation of available information that might be relevant to endocrine disruption is being conducted to select candidate chemicals subject to testing to assess their endocrine disrupting effects on aquatic organisms.

Until March 2016, through the reliability evaluation, 64 chemicals were identified as candidates for testing. For 40 substances out of the candidate chemicals, *in vitro* assays were conducted to identify the suspected mode of action related to endocrine disrupting effect. Moreover 10 substances for which positive results were obtained by the *in vitro* assays were subjected to Fish Short-Term Reproduction Assay (OECD TG229) using medaka. Based on the results of the FSTRA, 9 substances were identified as obtaining estrogenic activity, and 8 substances were identified as obtaining possibility of adverse effects.

In 2015, two test guidelines, Medaka Extended One Generation Reproduction Test (MEOGRT, OECD TG240) and Larval Amphibian Growth and Development Assaay (LAGDA, OECD TG241) have been approved and these TGs will be used for Tier 2 testing. Additionally some of the test methods, such as *in vivo* assay to detect anti-androgenic activity of chemicals and *Daphnia* multigeneration test, have been developing and optimizing.

Ministry of the Environment, Japan, decided to continue the program for endocrine disrupting chemicals as EXTEND2016 as an about-5-year proguramme in 2016. Hazards to human health identification of endocrine disruptting chemicals and international cooperation are big issue for the next proguramme.

Percellome Project with Special Reference to the Concept of "Signal Toxicity": Single Exposure Studies and a Newly Designed Repeated Dose Studies Introducing Baseline Responses and Transient Responses with Possible Link to Epigenetics

Jun Kanno, Japan Bioassay Research Center, Japan

Supported by the Ministry of Health Labour and Welfare, Percellome Toxicogenomics Project, using fewer animals exposing to lower doses for one time or short period of time, was initiated in 2001, aiming at mechanistically reinforcing the "safety (uncertainty) factor" used for the extrapolation of animal data to humans, and eventually replacing and making the process *in silico*. The project was designed not to miss any unpredicted toxicity. For this need, a normalization method designated as "Percellome[1,2], was developed for microarrays and Q-PCR to generate absolute copy numbers of mRNAs per one cell (in average). Quantified mRNA data of mouse liver (4 time points x 4 dose levels, n=3, 48 per organ per chemical) are obtained on more than 100 chemicals. Now the project includes studies on multi-organ relationship, low concentration inhalation, repeated dosing, etc. Data are visualized in 3D surface graphs (time x dose x mRNA copy number per cell) of each probesets of Affymetrix MOE430 2.0 GeneChip and subjected to comprehensive analysis by a series of in-house software. Here, we would like to report on the case studies on estragole[3] and pentachlorophenol[4], discovering unreported networks of PPAR-alpha and interferon signaling networks, respectively. Further strategy, including systems biology (Garuda Project[5]) will be briefly presented as well.

Percellome Project was primarily designed for the comprehensive drawing of gene network(s) in a time- and dose-dependent way after a single oral dosing of a chemical. The dose of each test chemical was determined by the intensive dose-finding study, and the highest dose was set to a level ("signal dose") that does not induce morphological changes (macro and micro) and clinical symptoms at 24 or during the first 24 hours post administration. Consequently, "phenotypic anchoring" was not considered as a tool for the transcriptomic data analysis. Along with the adoption of "Per cell" normalization strategy, use of gene knockout mice were considered for objective analysis of the gene network. It was expected that the gene network located downstream of the knocked out gene will be highlighted as its "shadow". Indeed, for example, when aryl hydrocarbon receptor knock out mouse (AhRKO) was challenged with 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) or 3-methyl cholanthrene (3-MC) and compared with wild type mice data, a group of genes including those known to be located downstream of AhR are silenced. On top of that, the gene list was larger than that of known downstream genes.

During conducting such analysis, we came up with an idea of a new concept of repeated dosing; the "chemically-induced transgenic state". It was considered that this concept should allow us to compare the responses of repeatedly dosed mice with that of the KO mice by challenging with a same test chemical. A series of trial studies are performed with a protocol as follows; all 48 mice were given a same amount of chemical "A" for up to 14 days by oral gavage, and then given, on the next day, a single gavage of the same chemical "A" at a dose of 0 (vehicle control), low, middle or high dose (in the range of "signal dose" mentioned above for single dose studies) and sampled at 2, 4, 8 and 24 hours thereafter for transcriptomic analysis (designated as [14+1] study). Compared with mice only receiving single gavage (designated as [0+1] study), we found that repeated dosing induces two types of responses on gene expression, i.e. baseline response and transient response. In general, when the baseline (vehicle control group) goes down (up), the transient response is attenuated (exaggerated). Further analysis on the data, including those from [4+1], [2+1] and [1+1] studies, and "A" + "B" studies using *in silico*

method on the upstream events will be discussed for the understanding of molecular basis of repeated exposure including possible epigenetic mechanisms.

The design and methods used in Percellome Project is published and the database will be widely available to the public soon via the Garuda Platform, one of the Open Biology activities.

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5. http://www.garuda-alliance.org/

DISCUSSION AND Q&A

- A participant asked whether the epigenetic change Dr. Jun Kanno noted the response was stable.
- Dr. Robert Kavlock asked whether Dr. Kanno determined the key initial signalling pathways. Dr. Kanno responded that he is currently analyzing the data for key signalling pathways and determining the AOPs.

Integrated Risk Assessment Methodology for Endocrine Disrupting Chemicals (IRAMe)

Kiyoung Lee, Seoul National University, Republic of Korea

Endocrine disrupting chemicals (EDCs) are emerging chemicals with possible adverse health effects from exposure to chemicals that can interfere with the endocrine system. The EDCs can be exposed through various exposure media and consumer products. With introduction of more chemicals with potential endocrine disrupting function, management of those chemicals is needed.

In this research for EDCs regulation in Korea, we will introduce several specific steps that can be useful for accelerating risk assessment.

1.) Prioritization of EDCs
With non-specific definition of EDCs, many organizations have their own list of EDCs. We developed prioritization method to identify priority EDCs using existing databases. The prioritization methods included human exposure, toxicity, and social concern. In addition to identification of priority EDCs from existing database, we are conducting biomonitoring to identify emerging EDCs. Based on prioritization and biomonitoring, we are constructing priority and emerging EDCs list.

2.) Toxicological application
Next step is to develop toxicological information of the EDCs. Although a few EDCs have toxicological information, majority of them do not have toxicity information especially endocrine

related effects. We are applying two approaches. 1) bioinformatics analysis of pre-existing data generated from toxicogenomic studies, and 2) *in vitro* toxicity comparison experiment.

Toxicogenomics are used to screen potential endocrine disrupting effects using existing database. For phthalate, NCBI GEO database was utilized. *In vitro* toxicity comparison is developed to determine relative potency factor based on well-known EDC.

3.) Integrated risk assessment for EDC chemical class
We performed the integrated risk assessment, at first, for phthalates and bisphenols, and will extend EDCs of target through biomonitoring in urines of susceptible population and chemical analysis of their surrounding environmental samples including indoor dust and air. We develop tentative reference dose (tRfD) which is the relative factor against DEHP, for emerging and alternative EDCs because their reference toxicity values are not developed yet, by conducing the comparative *in vitro* test using 3 different cell lines of H295R, MVRN, and GH3 (tier 1) and *in vivo* embryonic zebrafish assay (tier 2). Consequently, we can find the major source point for management strategy of not only conventional but emerging alternative EDCs.

DISCUSSION AND Q&A

- Dr. Tina Bahadori asked if Korea is still considering developing a national biomonitoring program. Dr. Kiyoung Lee explained that the Korean government has two national biomonitoring programs, one is run by the Korean agency and is similar to the US Centers for Disease Control and Prevention (CDC) and the other is run by the Ministry of the Environment. Dr. Lee pointed out that he did not have access to those biomonitoring data. He explained the problem is that the national biomonitoring programs focus on well-known compounds (e.g., heavy metals), but EDCs (e.g., phthalates) were not included in that process, though he hopes that the government funded his research with the intention of eventually including EDCs.
- Dr. Philippe Hubert asked how the Korean population reacted to the fact that their regulatory process was based on risk versus hazard. Dr. Lee noted that the general Korean population was very interested health, but the regulation and implementation of this program was promulgated under the Korean Environmental Risk Act, which specifically requires the implementation to be based on risk assessment and not hazard identification.
- Dr. Stan Barone asked how well EDC methodology was able to characterize the source attribution for exposure assessments, particularly for phthalates. Dr. Lee noted the difficulty of identifying all the potential sources of exposure and responded that, unfortunately, Korea does not have source information for phthalates.
- Dr. Bahadori asked if Korea could ask industry for the composition of their products under K-REACH. Dr. Lee explained that requesting composition of products was already part of the questionnaire that they send to companies, but regulations do not require industry to provide that information so industry often does not respond. Dr. Lee added that product composition information is also available through the Chemical Association, but industry has yet to approve its release.
- One participant noted the 88 pesticides that fall under Korea's current regulations and asked if Dr. Lee plans to use his integrated risk assessment to assess those pesticides. Dr. Lee explained that pesticides are regulated by the Ministry of Agriculture and fall outside of his purview at the Ministry of Environment.

Current Chemical Management and Prioritization in Taiwan

Steve (Yichen) Lin, Safety and Health Technology Center, Taiwan

The Occupational Safety and Health Act (OSHA) governed by the Ministry of Labor (MOL) and the Toxic Chemical Substances Control Act (TCSCA) governed by Taiwan's Environmental Protection Administration (Taiwan EPA), and several other regulations have been amended or developed to foster the safer use of chemicals to protect human health and environment. In 2009, Taiwan's MOL has incorporated relevant information nominated by industries and stakeholders to establish the very first national inventory, Toxic Chemical Substance Inventory (TCSI), which was sequentially announced in December 2014. The second edition of TCSI also has been officially released in August 2015. The TCSI lists over 100,000 chemical substances, including the existing chemical nomination held by the MOL before 2014 and another 7,500 substances received and reviewed by Taiwan EPA while implementing the latest existing chemical nomination in 2015. This TCSI has become the cornerstone for further chemical management modernization in Taiwan. Moreover, it distinguished the existing chemical substances from new chemical substances within the registration scheme under both TCSCA and OSHA to obtain chemical safety information similar to EU REACH dossier. The strategies for MOL are to manage chemicals operated in workplace with unreasonable risk will be assigned as Control Chemicals in workplace. MOL are performing the screening step for chemicals' data collected from Priority Management Chemicals reporting process, and further tier 1 and tier 2 assessment will proceed to determine the different designated chemicals as required for chemical exposure for labors. Taiwan EPA has started the tier 0 screening, and decided which chemical substances with adequate GHS classification will be assigned to high, moderate, and low risk categories. If chemical substances with insufficient GHS classification, then the industry challenge program will be performed to evaluate chemicals hazard identification. Due to data gaps and more detail information are required for hazard identification for MOL or Taiwan EPA, the new approach methods are evaluated to effectively fill in current lack data. Therefore, the all possible approach methods are potential to be considerate in further risk assessment in Taiwan chemical management programs.

DISCUSSION AND Q&A

- Dr. Tina Bahadori asked for further clarification on Taiwan's "REACH" regulation. Dr. Steve Lin explained that Taiwan is trying to implement a single regulation similar to REACH that would regulate priority chemical substances and new chemical substances differently. He noted that each ministry has a different set of criteria, so the specific methods depended on the specific ministry to which he reported. Dr. Lin added that the guidelines may change to complete prioritized work.
- Dr. Robert Kavlock asked whether Dr. Lin was able to request data on the 450 priority chemicals. Dr. Lin confirmed that importers and manufactures must satisfy the data requirements for each component of the chemicals they use, which includes submitting data on priority chemicals. Dr. Kavlock followed by asking if those data are confidential. Dr. Lin explained that the data submitted are publicly available, except for the data on new substances, for which only certain information is released.
- Mr. Mike Rasenberg asked to what extent the requests for data from industry needed to be substantiated and if those requests could be challenged. He also asked if the information gathered includes chemical structures and if companies using the chemicals could submit their IUCLID files. Dr. Lin answered the first question by noting numerous challenges, which have underscored the need to determine a better way to request data. Mr. Rasenberg asked if industry can submit IUCLID or similar files to Taiwan's regulatory bodies, and Dr. Lin clarified that IUCLID or similar files can be submitted for existing chemicals, but not new chemicals.

Introduction to Agency for Science, Technology & Research (A*STAR)

Kenneth Lee, Agency for Science, Technology & Research, Singapore

The Agency for Science, Technology and Research (A*STAR) is Singapore's lead government agency for catalysing and supporting industry development. Pharmaceuticals, Biologics, Consumer Care, Food and Nutrition, and Specialty Chemicals are some of the industries that are well-established or have a growing presence in this city-state in the heart of South-East Asia, which has a market size of US$1.9 trillion. Multinationals like Procter & Gamble and Nestlé have major R&D centres in Singapore and are partners of A*STAR, which has 18 research institutes and 4,600 scientists spanning the life sciences, chemical sciences, engineering, and modelling and computational sciences.

Within a framework of responsible innovation, we are in the initial stages of building a programme to help advance safety science and health research with innovative, non-animal tools. The programme will draw on A*STAR's multidisciplinary capabilities to address the growing need for more reliable, robust, and predictive methods for safety and efficacy testing. We have recently embarked on a partnership with US EPA. Specifically, we are collaborating with US EPA's National Centre for Computational Toxicology (NCCT) on three topics around kidney, liver and developmental toxicity. We have also begun engaging proactively with regulatory agencies in Singapore and in the near future will broaden our outreach to other ASEAN countries. We welcome the opportunity to collaborate with other like-minded organisations.

DISCUSSION AND Q&A

- Dr. Warren Casey asked if Dr. Kenneth Lee is comparing standard, approved OECD cell lines to determine if there are differences in skin sensitization between ethnicities. Dr. Lee explained that his skin model work is in the very early stages. He has a skin cell bank and is in the process of collecting skin cells from Chinese, Southeast Asian, and Indians to construct 3-D skin models.
- Mr. Mike Rasenberg noted that Singapore's regulations focus on classification, labelling, and safety data sheets, but do not necessarily have registration requirements. He asked how Dr. Lee's work feeds back into the determination and implementation of safer chemicals, but as answered previously, A*STAR works mainly with industry partners to develop the tools and NAMs that enable them to select safer chemicals during the product development process.
- Dr. Tina Bahadori asked if Singapore has more access to chemical formulation information because of their relationship with industry. Dr. Lee responded that Singapore has gained insight into chemical composition due to collaborations with industry. He noted, for example, personal care companies have shown interest in his skin sensitization models and they have expressed interest in validating the work together. The ultimate goal is build models that will be useful to stakeholders in the future. Dr. Bahadori followed by asking whether Singapore could leverage their relationship with industry to share information more transparently, and Dr. Lee responded that he would like to work together to increase publically available data.

Integrated Approaches to Testing and Assessment (IATA) Case Studies Project

Robert Diderich, OECD

In an effort to gain experience in utilising Integrated Approaches to Testing and Assessment (IATA) in various regulatory contexts, the Hazard Assessment Programme at the OECD commenced an "IATA Case Studies Project" in 2015 with the objective to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies. It is envisioned that case studies within this

project could be used as vehicles for further exploring the application and combination of AOPs, HTS, toxicogenomics and other *in vitro/in vivo* data. Information from this project will be made publicly available (http://www.oecd.org/env/ehs/risk-assessment/hazard-assessment.htm). Thus far countries and other stakeholders submitted and reviewed 4 case studies in 2015, and developed a considerations document of the learnings from the case studies. An additional 5 case studies are currently under review in 2016. Submission of case studies for 2017 is encouraged.

2015 Case Studies:

- *In Vitro* Mutagenicity of 3,3' Dimethoxybenzidine (DMOB) Based Direct Dyes [Canada and United States]
- Repeat Dose Toxicity of Substituted Diphenylamines (SDPA) [Canada]
- Hepatotoxicity of Allyl Ester Category [Japan]
- Bioaccumulation Potential of Biodegradation Products of 4,4'-Bis (chloromethyl)-1,1'-biphenyl [Japan]

2016 Case Studies:

- Repeated-Dose Toxicity of Phenolic Benzotriazoles [Japan]
- Pesticide Cumulative Risk Assessment & Assessment of Lifestage Susceptibility [United States]
- 90-Day Rat Oral Repeated-Dose Toxicity for Selected n-Alkanols: Read-Across [ICAPO]
- 90-Day Rat Oral Repeated-Dose Toxicity for Selected 2-Alkyl-1-alkanols: Read-Across [ICAPO]
- Chemical Safety Assessment Workflow Based on Exposure Considerations and Nonanimal Methods [JRC/BIAC]

DISCUSSION AND Q&A

- Mr. Jean-Lou Dorne asked how to assess variability and uncertainty in QSARs. Mr. Diderich explained that countries aimed to qualitatively determine how uncertainty may affect their regulatory decision. Mr. Dorne raised his concern that uncertainty and variability can be misinterpreted, and Mr. Diderich agreed. Mr. Diderich stated it may be more useful for scientists to determine if uncertainty poses a substantial issue and, if so, to attempt to reduce it to ensure regulators feel comfortable using the results.
- Ms. Lidka Maślankiewicz noted that these case studies can help to determine the aspects of case studies more generally that are relevant to regulators in terms of accepting the results. As an example, she pointed to an OECD project in which clear method definitions and explanations of result interpretations decreased the concern over uncertainty. She stated that the workshop participants could conduct another case study or more clearly define the case study approaches.
- Dr. Maurice Whelan stated that QSAR validation principles and reporting formats have been developed and wondered if attendees were generally unfamiliar or uncomfortable with such computational methods. He elaborated that computational-based NAMs can inform decisions and the predictive performance of models has greatly improved. Dr. Tina Bahadori responded that uncertainty within the field may stem from several issues. First, there is constant redirection of the next gold standard after animal testing. She also underscored uncertainty among the scientific community as to how barriers to modelling will be overcome if QSAR methods are not appropriately developed. Mr. Diderich responded that the key to success will be to build tools that reflect risk assessors thought processes. Dr. Tala Henry replied that she uses QSARs daily and did not agree with the barriers others described. Mr. Diderich clarified that the comments related to QSARs underscored the need to better describe the methods and interpretation of results. Mr. Diderich agreed with Dr. Henry that problems with QSAR can be addressed sufficiently by further describing the methods rather than using a different method.

Mr. Matthew Gagné agreed that the issue is not that QSAR methods are not accepted, but that they are not well described. Dr. Maślankiewicz agreed, noting that the criticism that QSAR methods do not have much experimental value usually stems from the need to better report the methods. Since there is usually no problem when those methods are reported well.

The Frank R. Lautenberg Chemical Safety for the 21st Century Act

Jeff Morris, US Environmental Protection Agency, USA

On June 22, 2016, President Barack Obama signed into law the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amends the Toxic Substance Control Act (TSCA), the primary US chemicals management law. The amended TSCA includes such improvements as a mandatory requirement for US EPA to evaluate existing chemicals with clear and enforceable deadlines, and a new risk-based safety standard. It also includes important provisions for alternative testing and considering susceptible populations.

DISCUSSION AND Q&A

- Dr. John Vandenberg asked what type of risk metric will be required by the updated regulation (e.g., exposure, maximum individual risk, or population risk). Mr. Jeff Morris explained that the law states that regulatory decisions cannot include non-risk factors, so US EPA plans to apply traditional benchmarks to determine acceptable levels of risk.
- Dr. Kenneth Lee asked if US EPA is able to issue an order to another agency or private company to conduct the testing. Mr. Morris responded that testing orders can be issued to manufacturers, importers, or processors of the chemical substance.
- Ms. Christine Norman asked if US EPA can use the test order results to inform prioritization, and Mr. Morris replied that the Agency can do that.
- One participant asked if and how US EPA can inquire about exposure to specific populations or during specific use scenarios. Mr. Morris explained that US EPA is developing generic information requests to survey industry for this type of information. The participant asked how a specific chemical within a mixture is determined to be the relevant exposure. Mr. Morris responded that developing generic scenarios will be helpful to address this issue.
- Another participant asked if US EPA anticipates asking industry for exposure testing and to develop approaches to determine adverse outcome pathways. Mr. Morris explained that nothing precluded US EPA from requesting exposure information and also noted significant data gaps.
- Dr. John Bucher asked if US EPA plans to negotiate with industry on alternative assays, or has the authority to simply direct that a specific assay be performed. Mr. Morris explained that the US EPA will likely discuss the alternative methods with industry as it will take time for these methods to be widely accepted.

Case Example for Use of High Throughput and Computational Approaches in Decision Making for Endocrine Disruption Potential

Stan Barone[1] (presenter), Kristan Markey[1], Carolina Pinto[2], Scott Lynn[1], Seema Schappelle[1], and Sharlene Matten[1]

[1]Office of Science Coordination and Policy, OCSPP, US EPA and [2]ORISE Fellow, USA

The Endocrine Disruptor Screening Program (EDSP) was established under authorities contained in the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act (SDWA) amendments. As mandated by these statutes, the EDSP develops a screening program to determine whether certain substances may

have endocrine activity in humans and wildlife. US EPA has developed a two tiered approach for screening chemicals and pesticides. The Tier 1 battery is used to identify substances that have potential to interact with the estrogen, androgen or thyroid hormone pathways. The Tier 2 tests identify and establish dose response information for adverse effects for substances identified in the Tier 1 screening.

Additionally, EDSP is incorporating ToxCast high throughput screening data and computational models in the prioritization and screening of a chemical's potential to interact with the endocrine system in humans and wildlife for a portion of the Tier 1 battery. This approach will allow nearly 20 times the current number of screenings to be performed while nearly eliminating animal testing, allowing the program to meet its goals with a relatively level budget. In coming years, OCSPP plans to expand this concept to screen for other endocrine and potentially non-endocrine endpoints. This technological breakthrough can massively expand OCSPP's ability to screen and assess chemical safety, including existing chemicals currently under evaluation, prior to seeking data from industry partners.

The EPA's EDSP is expanding the use of high-throughput assays and computational tools to prioritize and screen chemicals for potential endocrine bioactivity and exposure; in particular, the estrogen, androgen, or thyroid hormone pathways in humans and wildlife. The expanded use of these alternative testing methodologies increases the output of screening results and allows for greater coverage of the endocrine system. The vanguard efforts of the EDSP related to endocrine bioactivity will allow the OCSPP programs to apply these alternative testing methodologies to EDSP and non-EDSP-related evaluation of developmental neurotoxicity, immunotoxicity and other toxic effects.

These new approaches have been successfully used in a number of decision contexts. Most notably, the screening and prioritization of chemicals and pesticides through EDSP has resulted in the refinement of Tier 1 EDSP *in vitro* and *in vivo* tests and the availability of alternative testing procedures for the estrogen pathway. Other aspects of current implementation of alternative data have been leveraged in the development of AOP frameworks for prediction of adverse outcomes and use in weight of evidence analyses. However, key challenges remain in addressing acceptance of alternative data in different decision contexts. Some of these challenges relate to interpretation and extrapolation issues for alternative approaches including coverage of biological space, coverage of chemical space, false negatives associated with signal detection and metabolic potential, and *in vitro* to *in vivo* extrapolation.

The views expressed in this abstract do not necessarily reflect US EPA policy.

DISCUSSION AND Q&A

- Dr. Tara Barton-Maclaren asked if US EPA plans to conduct a similar high throughput assay for substances that are not as easily tested, as that list of chemicals is large. Dr. Stan Barone responded that ORD currently discussing how to conduct those tests. Dr. Rusty Thomas added that US EPA hopes to test several volatiles for bioactivity in the next 12–18 months.

California's Approach to Evaluating and Incorporating New Methods in Prioritization and Risk Assessment

Gina M. Solomon, California Environmental Protection Agency, USA

California's data needs span a wide range of decision contexts, including: (1) Prioritization of chemicals for our Biomonitoring California program; (2) Quantitative risk assessment of pesticides, drinking water contaminants, waste sites, and toxic air contaminants under various statutes; and (3) Selection of chemical-product combinations for alternatives analysis under our Safer Consumer Products (SCP) Program. Recognizing the potential importance of new alternative methods (NAMs) for toxicity and exposure evaluation, the California Environmental Protection Agency (CalEPA) initiated in 2013 an intra-Agency effort to evaluate the utility of new alternative data in our programs.

Toxicologists and environmental scientists within CalEPA are working in teams to develop subject matter expertise in emerging methods, including ToxCast, Tox21, and various chemical use and exposure tools. We have published papers on some of our initial case studies, including on pesticides and phthalates, and a paper referring to its use in biomonitoring prioritization. Our Department of Pesticide Regulation is incorporating NAM data summaries into risk assessments to support weight-of-evidence determinations for pesticides, and the Office of Environmental Health Hazard Assessment is similarly using the data as part of mechanistic evaluations and in support of hazard trait and dose response assessment. SCP scientists are using emerging tools for chemical use and exposure potential to aid in selection of chemical-product combinations for evaluation.

NAMs have significant potential to aid in prioritization. For example, our Office of Environmental Health Hazard Assessment has created structural and functional groups of chemicals that have been successfully prioritized for biomonitoring by our independent Scientific Guidance Panel. Examples of such groups include p,p'-bisphenols; non-halogenated aromatic phosphates; and synthetic polycyclic musks. NAM data have potential utility for supporting the identification and prioritization of chemical groups, and to support read-across evaluations for hazard trait identification and dose-response.

Greater detail about chemical functional uses, chemical roles throughout the supply chain, and exposure potential would be highly beneficial for prioritization, but such data are very limited. Exposure NAMs are generally not as developed as toxicity evaluation tools, but are nonetheless important to us as end-users.

In risk assessment, we have encountered some challenges with the new data, including failure of the alternative methods to identify key, established toxicity endpoints for some of our test-case pesticides, and limited ability to group phthalates according to common toxicity characteristics or provide relevant information on mode of action. These challenges suggest a need for caution prior to relying on alternative methods, especially when these methods are used to support a finding of absence of a hazard.

DISCUSSION AND Q&A

- Dr. Maurice Whelan inquired if California performs impact assessments for proposed policies. Dr. Gina Solomon responded that California considers the economic impact of each regulation. When economic impact is anticipated to exceed $50 million, a full assessment of the projected impacts on the California economy is conducted. She also noted that the economic impact analyses are uncertain for several reasons, including that they are forward-looking and that some programs have not yet initiated regulatory action. Economic impacts are also difficult to monetize. As an example, she pointed to Proposition 65 (Prop 65), which requires businesses that expose the population to a chemical that is known by the state of California to cause cancer or reproductive harm to first warn consumers. The economic value of Prop 65 is difficult to quantify, but the benefit has been product substitution. Dr. Solomon stated that the quiet substitution of chemical products driven by Prop 65 has underscored the need for systematic alternative analyses.
- Dr. Stan Barone followed by asking whether CalEPA has conducted any analyses considering regrettable substitutions following Prop 65. Dr. Solomon explained that Prop 65 does not require industry to provide any information about the replacement chemical(s), so regrettable exposures are difficult to determine.
- Dr. Phillippe Hubert asked whether the case studies considered epidemiology and traditional toxicology data in addition to the high-throughput *in vitro* data. Dr. Solomon replied that both the pesticide and phthalate case studies considered all available epidemiology and animal data.

She noted for endosulfan specifically, there is good agreement between all of the different data streams regarding both the estrogenic effects as well as the mode of action.

- Dr. Warren Casey responded that comparing *in vivo* and *in vitro* data is similar to comparing apples and oranges, noting that the two models are held to different standards. Dr. Solomon had referenced a positive estrogenic response in the *in vivo* model for endosulfan, but he questioned whether existing uterotrophic studies have shown similar effects. It will be critical to impose the same quality metrics around *in vivo* data that are currently demanded of *in vitro* data.
- Another participant asked whether the discrepancy between the *in vitro* and *in vivo* data in the pesticide case study may have been related to testing endosulfan versus the biologically active ingredient and if the analysis considered mode of action. Dr. Solomon responded that CalEPA picked the specific pesticides for the initial case study because there was extensive data on the endpoints and mode of action, so the goal was to determine if there was concordance between the data streams in a way that could be used across chemicals.

Closing and Considerations for Tomorrow

Robert J. Kavlock, US Environmental Protection Agency, USA

Dr. Robert Kavlock reiterated that the goal for the first day of the meeting was to introduce the participants to the various risk assessment efforts around the world. He noted that the demand for alternative methods that help increase the pace of risk assessment is apparent. Several presentations described case study applications of these methods. He noted a few themes that recurred throughout the presentations, which included questionable confidence in negative results, determining an acceptable level for positive responses, increasing consideration of exposure (as opposed to focusing solely on hazard), and developing collaborative databases to avoid redundant research. He stated that during the second day of the meeting, the participants will hear about additional case study applications, after which they will discuss future collaborations and next steps. He thanked the attendees for their participation.

Review of Activities of Day 1

Robert J. Kavlock, US Environmental Protection Agency, USA

Dr. Robert Kavlock reconvened the meeting for Day 2 by noting the previous day's purpose was to increase awareness of on-going international activities related to chemical risk assessment. He quickly reviewed the agenda for the day and underscored the aim: to commit to moving forward together on chemical risk assessment and to determine a vision to do so.

Use of All Available Data in Accelerated Chemicals Assessment

John R. Bucher, National Toxicology Program, USA

Background: Advances in toxicology including alternative species, Tox 21, TSCA reform, and application of systematic review methods to environmental health information offer new opportunities to use diverse data in new ways for public health decisions. The National Toxicology Program (NTP) response to a brief contamination of the Charleston, West Virginia (WV) drinking water supply resulting from a chemical spill into source water provided a unique dataset to predict few health concerns.

Methods: Coal cleaning chemicals spilled into the Elk River were subjected to SAR (structure-activity relationship) analyses, and studied in bacterial mutagenicity, Tox21, *C. elegans*, zebrafish, and for teratology, genetic toxicity, and toxicogenomics in rats.

Approach: Results were modeled using benchmark dose analyses and compared to the drinking water advisory levels established by CDC at the time of the spill.

Results: SAR suggested concerns for development and irritancy. Results of Tox21, *C. elegans*, zebrafish, and genetic toxicity were largely negative. Toxicogenomics showed a lack of gene induction in pathways of toxicity concern in liver and kidney of rats. Teratology studies suggested only lower birth weights in rat pups.

Conclusions: Public health concerns were mainly for exposures to pregnant women in the Charleston area. The NTP data suggested few effects other than lower birth weights in rats. The state of WV subsequently studied birth weights in the Charleston area during the years before and following the spill. No effects were observed. Increasingly toxicology will need to balance potential human exposure levels with data from rapid studies establishing exposure levels that do not cause an effect, rather than the slow, exhaustive demonstration of levels that do cause apical outcomes. Systematic review methods, although not used in this case, can in the future help in focused problem formulation, comprehensive literature analysis and data integration across multiple evidence streams to use all available data.

DISCUSSION AND Q&A

- Dr. John Vandenberg asked if NTP would have concluded MCHM (4-Methyl-cyclo-hexane-methanol) is not of concern had the agency had not had the results of the 28-day rat study to rely upon, and how new study types are used to assess risk. Dr. John Bucher responded that the SAR data predicted the developmental effects, which was a signal from the beginning, and added that the 5-day toxicogenomic data provided similar assurance. He added that in this situation, though, it would not have been sufficient to conclude no risk based on high-throughput data alone.
- Dr. Rusty Thomas asked about the exposure protocol for the developmental study. Dr. Bucher informed the group that the rats were exposed via drinking water (note added in proof, the

studies were actually performed using oral gavage). Dr. Thomas then asked if NTP would do anything different in hindsight. Dr. Bucher responded that the response will always depend on the chemical and the overall risk context. Predicting the toxicity of the material is critical, and the 5-day studies give good insights. Using a tiered-approach and taking advantage of rapid assessment methods, where possible, will also be important.

- Another participant asked about the dose-response curve, specifically regarding the separation between bioactivity and toxicity. Dr. Bucher replied that this difference is typical. Scientists are still determining whether short term genomic studies are predictive of long term effects, but he noted the general rule appears to be a 10-fold reduction in concentrations from the phenotypic to genotypic response.
- An attendee asked whether the researchers had considered bioinjection as the exposure method to assess a worst-case scenario and if they had considered any toxicokinetic work. Dr. Bucher stated that the group did not consider bioinjection (intraperitoneal), though there was some interest in inhalation studies. Related to the toxicokinetic data, Dr. Bucher responded that there were no data for humans, so they relied on backward calculations based on the *in vitro* well concentrations and the drinking water concentrations in the animal studies.

Development of the RapidTox Decision Support Tool for 21st Century Chemical Risk Assessment

Russell Thomas, US Environmental Protection Agency, USA

The path for incorporating new approach methods and technologies into chemical risk assessment poses a diverse range of challenges including delivering the data in a useful way to risk assessors. The goal of the RapidTox project is to integrate a range of information related to chemical properties, fate and transport, hazard, mode-of-action, and exposure through an interactive on-line decision support tool in order to enable screening-level assessments to be performed for hundreds to thousands of chemicals. The data will be delivered as a tiered approach where traditional, high-quality data will be provided when available with lower tier data, including new approach methods, provided when higher tier data is not available. The RapidTox tool is being developed in close partnership with regulatory partners using a series of case studies. The first case study will use the tool to prioritize non-food use pesticidal inert ingredients for additional study. The second case study will quantitatively estimate screening level toxicity values with associated uncertainty for data poor chemicals at Superfund sites. The presentation will cover development of the RapidTox tool as an example of what chemical risk assessments could look like in the 21st century.

DISCUSSION AND Q&A

- Multiple participants asked about feedback from users related to their experience using the RapidTox tool. Dr. Rusty Thomas replied that RapidTox was intended to be interactive. The features are added and edited iteratively in 2-week intervals, and the team finds this regular feedback from the program partners helpful. He also mentioned that they receive feedback from users of other dashboards that are available more broadly and are constantly improving the tool using on-going feedback. US EPA would like to integrate RapidTox with the ToxRef database and ECHA's *in vitro* database, if approved. He added that US EPA would also like to make the tool public eventually.
- Dr. Tomasz Sobański mentioned the impact of the difference between testing materials as compared to what is available on the market, and asked how RapidTox addresses this uncertainty. Dr. Thomas replied that in ToxCast21, chemicals from multiple sources are analytically characterized in terms of purity, and the NAMs are relatively economical so multiple mixtures can be tested. He conceded that this does not fully address the issue of proprietary

market mixtures. Another participant agreed that it is difficult to determine which chemicals were tested, but added that often databases do not agree in terms of how they reference a chemical. Dr. Thomas acknowledged this issue and stated that RapidTox attempts to be clear about which chemicals were tested by curating chemical structures and identifiers.

- Another participant inquired about the design of workflows within the context of NAM development and a tiered decision making approach. Dr. Thomas responded that in the past, the workflow has used a waterfall design, where the assignment is given to the contractor and the finished product is delivered. In the new approach, RapidTox is improved iteratively and uses a modular system, which is flexible for use in a number of decision contexts. Another attendee added that it will also be important to record decision points, so others can replicate and compare across individual users and decision contexts.

Facilitated Discussion: What Do Regulators Need to Accelerate Risk Assessment?

Co-Chairs: Stanley Barone, US EPA, and Maurice Whelan, European Commission Joint Research Centre

Dr. Maurice Whelan, head of the Chemical Safety and Alternative Methods Unit at the European Commission Joint Research Centre (JRC), emphasized that the point of this was discussion, not necessarily solutions. Communicating criteria for successful incorporation of NAMs into various risk assessment contexts would be beneficial for everyone.

Dr. Stan Barone, Director of the Office of Science Coordination and Policy at US EPA, explained that scientists often struggle with how to translate research into something useful for decision-makers in a way that is transparent enough for stakeholders to understand the process. In order to make research defensible, scientists must consider these contexts. He also asked about feedback the other scientists get related to false positives and negatives, the epidemiologic and clinical contexts, integrating multiple data streams, transparency, and other issues. He emphasized the importance of integrating scientific research into a larger construct.

Mr. Mike Rasenberg, Head of the Computational Assessment and Dissemination Unit at ECHA, provided a few thoughts and questions intended to provoke discussion.

The moderators then opened up the floor for general discussion. The following observations, action items, needs, and points of discussion were covered:

(Divided into topic areas following the meeting)

1. Need for a culture change

- **Need to think about the fundamental need**. Protecting public health and the environment is the underlying mission for risk assessment. Accelerating risk assessments is beneficial to industry and to regulators, but risk assessors must determine if NAMs protect public health.
- **Three disconnects to address**.
    1. Toxicology and life sciences
    2. Industry and authority: risk assessment authorities can leverage industry's need to accelerate the pace of risk assessment to quickly screen products.
    3. Geography: creating a risk assessment network between the US, Canada, Europe, and Asia
- **Need to embrace qualitative approaches**. Often times the risk assessment community can become fixated on quantitative values. There is value in making good binary decisions, and then moving on to semi-quantitative or quantitative approaches.

- **Need to think about 2020 and beyond**. This community should not miss out on progress by focusing too much on goals to accomplish by 2020. Risk assessors should focus on big picture goals.

2. Need for a champion for strategic vision and coordination

- **Need to facilitate continued engagement.** In order to have an international risk assessment network, a facilitator is necessary. OECD appears to be the best option.
- **Need for third-party facilitation and empowerment**. Work with third-party organizations (like OECD) to develop a strategic approach to prioritize activities would be beneficial. They can take on a more proactive role to ensure high-priority activities are addressed. If there is a disconnect in priorities, this should be discussed as OECD member countries drive the action.
- **Need for a collective vision for strategic thinking and implementation.** This is a real opportunity to address broader public health issues (i.e., mixtures, cumulative exposures).
- **Need to articulate uncertainties and variability.** It will then be possible to highlight which ones are reduced by moving to a NAM-based system.
- **Need to encourage product stewardship approaches**. Industry has a front-line responsibility for classification.

3. Need for standardization of data and methods

- **NAMs and classification.** Rather than getting rid of existing classification approaches, new classification schemes using NAMs should be explored in parallel to existing approaches. The current classification scheme could be expanded to include contemporary biology.
- **Need institutionalization of methods**. The extent to which risk assessors can work toward institutional relationships and mutual acceptance of data will be useful.
- **Need a standardized internationally recognized set of assays** that risk assessors can ask industry to perform. Additionally, there is a need for more rapidly establishing the fitness-for-purpose of newly proposed assays since current validation approaches which assess between-laboratory reproducibility as a step towards the development of an international standard (e.g. OECD Test Guideline) requires multiple labs and years, which is not feasible for a large number of promising assays.
- **Need an assessment of OECD Guidelines.** It is necessary to evaluate what the guidelines do and do not provide for risk assessment, and where NAMs fit into them.
- **Need to put animal studies in context**. Animal studies should not be treated as the gold standard, and risk assessors should attempt to improve on them. NAMs should be able to assess risk better than animal studies. If animal studies continue to be used as a benchmark for success, risk assessments will fall behind and not make progress. Especially when considering the progress made in human disease diagnoses, which animal studies are not keeping up with. Instead of attempting to model animal bioassays, NAMs should focus on modelling the human disease process and human health outcomes.
- **Need to bridge animal data with NAM data**. An example approach is California's "hazard traits" system. CalEPA identified "hazard traits" of chemicals and provided that information to the public. They realized it was an opportunity to define what signals could be obtained from an animal test as compared to *in vitro* assays.
- **Need to think about performance-based standards**. They should consider flexibility versus prescriptiveness and what appropriate validation procedures might be.

4. Need for accessibility of data and methods

- **Need data for data-poor chemicals.** Most current work is focused on data-rich chemicals, and data-poor chemicals also need to be examined.
- **Need to enable move from research labs to commercial labs.** If regulatory agencies are going to tell chemical companies what tests they need to perform, the toolbox must be accessible in a commercial context. Transitioning from a research (e.g., results of case studies) to a practice context should be articulated in a more pragmatic way.
- **Need for a universal data platform/dashboard.** Having a common platform for data sharing between scientists and regulatory groups is an essential need. For example, US EPA is rolling out a database with ToxCast and reference chemical data, which is set up so users can input a chemical and get all the available information.

5. Need for increased confidence and understanding of NAMs and their use in decision-making

- **Need training** at every level, from risk managers to risk assessors.
- **Need a knowledge-based approach to translate or prepare NAMs for regulatory decision-making.** It is important for those who are writing regulations to become more comfortable with NAMs, and to understand how they fit into the risk assessment process.
- **Need to move away from interpreting NAM results through an endpoint paradigm/phenotypic responses**. It will be useful to use an alternative approach to NAMs, given that some exposure levels that do not produce a phenotypic biological effect.
- **Need to link data with knowledge on AOPs.** Linking a data platform to the knowledge base on AOPs and MOAs will be the next step and will help with the usability of results.
- **Need to integrate NAMs with epidemiology.** While NAMs may replace animal studies, they cannot replace epidemiologic studies. It is important to consider how to integrate and incorporate epidemiologic data to get a fuller picture of a chemical. NAMs can help us understand the context of epidemiologic data, and look at things in a more holistic fashion.
- **Potential for NAMs to be used as a supplement to risk assessment**. There are steps in risk assessment where new tools can be applied to supplement the standard risk assessment process. That is a potential place for NAMs to be introduced. The entire decision will not be based on them, but NAMs can be used to supplement data gaps.
- **Need confidence within decision-context.** Risk managers and regulators must be confident in the decisions they make. To achieve this, there must be a common understanding of the science behind data generation to interpret assays commonly across all agencies and to analyze data in a similar manner. Risk assessment results can be used differently in different legal contexts, but the science should not vary. Risk assessors should develop an approach for integrating and benchmarking *in vitro* data against *in vivo* data.

6. Need for progress

- **Small successes are the path to fundamentally change what is being done**. The risk assessment community needs to accumulate small successes (for example, a workshop of ICATM, the International Cooperation on Alternative Testing Methods, in October 2016 is taking OECD case studies for skin sensitization and trying to build an international consensus on the regulatory applicability and acceptance).

- **Need to identify "low-hanging fruit."** The risk assessment community should tackle some of the issues that are relative easy to resolve to create confidence in and lead to acceptance of NAMs. For example, promoting the notion that there is less risk if exposure is below a defined level of bioactivity, as in the BER context. This addresses regulatory decision making without connecting assessments to specific endpoints.
- **Shift from *in vivo* animal studies to NAMs needs to be done carefully, but can begin now.** NAMs should be introduced initially as a complement to, not replacement of, animal studies. The focus should be on their added value to the risk assessment process. In this context, the introduction of NAMs can start now.
- **Need to accelerate *in vitro*-to-*in vivo* (IVIVE) progress.** It is important to understand how *in vitro* studies compare to *in vivo* studies, and how to get from one to the other.
- **Potential for exposure data to move ahead and create a strong need for NAM toxicity data**. Three things make this possible: 1) development of non-targeted biomonitoring methods make it possible to identify chemicals not previously known, 2) strategies for direct potable reuse of water for drinking water raises questions about what is in drinking water, and 3) Google StreetView cars are currently collecting extensive air monitoring data in Los Angeles. These will drive risk assessors to quickly assess the toxicity of these new chemicals and exposure scenarios, which is exactly the scenario for which NAMs are intended. That is an opportunity to demonstrate the value of these approaches.

Facilitated Discussion: What Are the Chemicals of Common Interest Internationally?

Co-Chairs: Tala Henry, US EPA, and Kerry Nugent, NICNAS

Dr. Tala Henry, Director of the Risk Assessment Division in the Office of Pollution Prevention and Toxics (OPPT) at US EPA, opened the session on chemicals of international interest by reiterating the importance of case studies. She reminded the participants that the goal of the workshop is to identify a list of case studies and collaborations to work on into the near future. She noted that, ideally, the case studies will not only be scientifically meaningful, but will also be applicable in the regulatory system.

Dr. Kerry Nugent, Principal Scientist at NICNAS, urged participants to also think about which case studies might be of interest to the regulators themselves.

Mr. Matthew Gagné, Senior Chemical Evaluator in the Healthy Environments and Consumer Safety Branch of Health Canada, presented a master list of chemicals of interest that he and the Health Canada team assembled ahead of the workshop. Mr. Gagné explained that the spreadsheet contains a list of priority chemicals sent by each organization as well as substances listed on agency websites. The spreadsheet includes information on the substances physical and chemical properties, production volumes, toxicity data from animal and NAM sources, and information from previous risk assessments. He suggested four data scenarios on which the case studies could potentially be built:

- **Scenario 1**: a previous risk assessment has been completed, and NAMs are available.
- **Scenario 2**: no previous risk assessment has been completed. Classic toxicity data and NAMs are both available.
- **Scenario 3**: no previous risk assessment has been completed. Classic toxicity data are available, but NAMs are not.
- **Scenario 4**: no previous risk assessment has been completed. Neither classic toxicity data nor NAMs are available.

Mr. Gagné also demonstrated how to use the spreadsheet.

Dr. Henry and Dr. Nugent asked the participants if they were in agreement on whether case studies should be pursued, and the group emphatically agreed. The co-chairs then opened for questions from the participants.

Mr. Gagné received a question regarding how the source count was applied for EPA, and he informed the group that all data from US EPA were denoted as a single source, and specific offices were not counted as separate sources. Another question pertained to the example chemicals in each scenario and, in the instances that a class of chemicals was noted to have data available, whether this means that all chemicals in the class had data available or simply if data were available for the class combined. Mr. Gagné replied that for the phthalate example specifically, all 11 chemicals had data available.

One attendee applauded the effort, noting that the next step will be to apply the resource. He added that the group will need to determine the goal of the case study and agree upon which scenario to focus their efforts, but supported working on the new chemicals with fewer data. A number of other participants agreed, pointing to the need to prioritize determining how to use data poor chemicals in risk assessment. Another participant added that RIVM (The Netherlands National Institute for Public Health and the Environment) is currently conducting two case studies related to scenario 2 and questioned the value of a scenario 1 case study, unless the goal is to validate the NAMs.

Another attendee suggested a hybrid approach, in which NAMs data for a data-poor chemical are compared via read across to a data-rich chemical and the matrix is ultimately used to inform prioritization.

Dr. Thomas Burke commented that the group has a wonderful opportunity to strengthen the application of NAMs data across the various scenarios, but he cautioned against focusing on the data-rich scenarios.

One participant pointed out the relative lack of substances in scenario 4 and suggested that the group may need to alter its grouping criteria. Mr. Gagné responded that the group considered forward priority chemicals, which may have affected the size of the groups. Furthermore, industrial chemicals associated only with occupational exposures were not included.

Dr. Henry noted some consensus around focusing on data-poor chemicals, and shifted the conversation to the additional information that would be necessary to initiate the case studies. One attendee suggested expanding the list of chemicals to include TSCA chemicals and focusing on chemicals that regulatory agencies are interested in, though someone else cautioned against focusing on only a few classes of chemicals. Another participant suggested focusing on selection criteria, and another attendee suggested that one of the criteria be that the chemical class contains a few data-rich chemicals and several data-poor chemicals, offering siloxanes and non-halogenated aromatic phosphates as specific examples. Other participants underscored their interest in endocrine disruptors and pesticides and/or their metabolites.

Dr. Nugent remarked that chemical class will be a criteria, perhaps the group should discuss specific outcomes of interest related to these classes. Participants identified organ toxicity, carcinogenicity, mutagenicity, and reproductive toxicity.

Facilitated Discussion: How Do We Develop Shared Case Studies to Address the Challenges?

*Co-Chairs: Tina Bahadori, US EPA, and Kenneth Lee, A*STAR*

Dr. Tina Bahadori, National Program Director of Chemical Safety for Sustainability at US EPA, opened the session by asking participants to suggest criteria to shape potential case studies. Participants asked for clarification on what was meant by criteria; Dr. Bahadori explained it meant something they could all gather behind, for example Health Canada's BERs have certain desirable characteristics. Members of the

audiences noted that developing common criteria may be difficult given the many differences between chemicals (range of hazards and exposures, level of priority, data-rich vs. data-poor, etc.). One participant suggested as a criteria that NAMs should be commercially available or easily reproducible.

Dr. Bahadori emphasized that the value of a case study is to demonstrate an application of NAM that, if successful, can be then provided to stakeholders as a method of generating data. The NAMs case studies will serve as a scientific proof of concept, contribute to real chemical assessments, and demonstrate that NAMs accelerate risk assessment. Additionally, the case studies provide a medium for international collaboration. She noted that the case studies could investigate existing NAMs or develop new methods. One participant reminded the group that the replacement of animal models with NAMs will not be one-for-one; for example, multiple assays may be needed to replace a single 90-day study.

The workgroup participants discussed methods of categorization of the case studies. Several attendees suggested categorizing by endpoint or adverse outcome pathway. Another participant suggested categorizing by approach: 1) prioritization and screening, 2) read across, and 3) risk assessments and weight-of-evidence, in order to determine how NAMs perform within each and to show how approaches can be implemented across a large group of chemicals. Others cautioned that this method could lead to research silos.

Participants then turned to the structure of the case studies. One approach discussed was to conduct a new assessment using only NAMs on a data-rich chemical and comparing the NAM results to the results produced by the traditional methods. Another option would be to focus on a data-poor chemical and start from scratch. Participants noted the spectrum of options between these two options. Some participants expressed hesitancy toward conducting case studies that would make comparisons with the existing data, and expressed interest in contributing new scientific knowledge. One participant suggested randomly sampling data-poor chemicals, testing them with several NAMs, and using the preliminary results as a method for determining next steps.

Dr. Kenneth Lee, Senior Director at A*STAR, asked the participants from Asian countries what would entice them to participate in the case studies. Attendees from Japan mentioned that more data sharing would be helpful. The Taiwanese participants stated that they could contribute data to a case study. The Korean attendees noted that the Korean Ministry of Environment is also open to sharing data. They also expressed interest in the data-rich exposure-based approach to case studies.

The group then discussed how to move forward with the case studies. The participants decided that groups would volunteer for "designer" case studies and submit proposals (including description and timeline). The proposals will be shared with all meeting attendees, who can then decide if they want to participate in the case study.

Case Study Proposals (topic details updated following the meeting)

- **Data-poor scenario** (Mike Rasenberg, ECHA)
- **Foods and pesticides** (Jean-Lou Dorne, EFSA and Jose Tarazona, EFSA)
- **Duck, duck, goose: Revisiting and updating chemical categorizations with new approach methods (NAMs)** (Tala Henry, US EPA and Daniel Chang, US EPA)
- **Examining In Vitro Bioactivity as a Conservative Point of Departure** (Rusty Thomas, EPA)
- **Safer choice for preservatives** (Kristan Markey, US EPA)
- **Case Study on EDCs** (Phillippe Hubert, INERIS)
- **Application of New Alternative Methods (NAMs) to Inform the Human Health Toxicity of Perfluoroalkylated Substances (PFAS)** (Lynn Flowers, US EPA)
- **Triaging Exposure Data and Modeling Needs for Exogenous Chemicals** (Jennifer Orme-Zavaleta, US EPA)

- **Linking Exposure to Toxicology Using Lead As A Case Study: Opportunities for Collaborative Data Sharing/Generation** (Jennifer Orme-Zavaleta, US EPA)
- **Aromatic amines** (Kerry Nugent, NICNAS)

One-page concept proposals for case studies are due in one month (**November 1st**) to Maureen Gwinn (**gwinn.maureen@epa.gov**).

Agreement on All Action Items, Timeline, and Leads

Co-Chairs: Robert J. Kavlock, US EPA, and Jeff Morris, US EPA

Mr. Jeff Morris, Deputy Director of OPPT at US EPA, expressed how impressed he was with the undercurrent of optimism at this meeting. He also expressed that NAM work has spent a long time in the research & development space, and this meeting was timely. The challenge now is to move NAMs into the regulatory decision making process. He noted that case studies strategically designed to achieve this will be beneficial.

Summary and Conclusion

Robert J. Kavlock, US Environmental Protection Agency, USA

Dr. Robert Kavlock concluded that the meeting produced a meaningful direction for the future to improve and accelerate the chemical risk assessment process. He emphasized that regulators must know what they should be regulating in order to best protect public health.

Dr. Kavlock divided the discussed activities into two categories:

1. **Foundational** – must be conducted first to take advantage of other activities.
    a. ***Data Platforms***: For chemicals (Health Canada's work), hazard data (OECD eChemPortal), etc. What action is needed to make this happen? Is there a group willing to work on this (Mike Rasenberg, Maurice Whelan, and Robert Diderich)?
    b. ***Classification Systems for NAMS***: There are systems for traditional toxicity data but not for NAMs. Is that something that can be done? What would it look like? What value would be added?
    c. ***Exposures***: There is the possibility for a foundational shift in how exposures are assessed (i.e., non-targeted screening for application of chemicals).
2. **Experimental**
    a. ***Data Generation***: It is critical to generate data not only in research but also in commercial venues.
    b. ***Case Studies***: It is necessary to explore how to make NAM case studies useful to regulators. Several topics related to how to develop the case studies were discussed: criteria, priority chemicals, endpoint focus vs. not, hot button controversial issue vs. not.

Dr. Kavlock then reviewed the case study proposals discussed previously. He reiterated that proposals for case studies are due in one month (**November 1st**) to Maureen Gwinn (**gwinn.maureen@epa.gov**), who will collate and distribute them. He emphasized that the case studies should not become national silos. The goal is international collaboration and engagement. Dr. Kavlock proposed reconvening as a group to discuss progress in the next calendar year. He reminded the attendees that the case studies are a purely academic exercise, as there are no binding legal constructs to the proposals.

Dr. Kavlock thanked everyone for their participation, and wished them a safe journey home.

APPENDIX A: AGENDA

DAY 1 – WEDNESDAY, SEPTEMBER 14, 2016

8:30 am **Registration**

9:00 am **Welcome**
Thomas A. Burke, PhD, MPH
Deputy Assistant Administrator, Office of Research and Development, US EPA

Jim Jones
Assistant Administrator, Office of Chemical Safety and Pollution Prevention, US EPA

Robert J. Kavlock, PhD
Deputy Assistant Administrator, Office of Research and Development, US EPA

9:30 am **Introductions**
Tour of the tables

Experiences of Participating Organizations

10:00am **The NICNAS Inventory Multi-tiered Assessment and Prioritisation (IMAP) Program**
Kerry Nugent, PhD
National Industrial Chemicals Notification and Assessment Scheme
Australia

10:30am **New Approach Methodologies to Support Canada's Chemicals Management Plan**
Tara Barton-Maclaren, PhD
Health Canada
Canada

11:00am **REACH and CLP and the Use of New Approach Methods Information**
Mike Rasenberg
European Chemical Agency (EChA)
European Union

11:30am **Connecting Exposure, Toxicokinetics and Toxicity: Towards Animal Free Risk Assessment in Food Safety?**
Jean-Lou Dorne, PhD
European Food Safety Authority
European Union

12:00pm **Computational Risk Assessment for Mixtures of Chemicals: The Case of Aromatase Inhibitors**
Philippe Hubert, PhD
INERIS, France

12:15pm	**LUNCH**
1:15pm	**Percellome Project with Special Reference to the Concept of "Signal Toxicity": Single Exposure Studies and a Newly Designed Repeated Dose Studies Introducing Baseline Responses and Transient Responses with Possible Link to Epigenetics** Jun Kanno, MD, PhD Japan Bioassay Research Center
	List of Chemicals in the Ministry of the Environment, Japan Progress of EXTEND2010. Results of Tier-1 Screening Assays for Candidate Substances, Aspect and Issues for Tier-1 Assessment and Tier-2 Testing Taisen Iguchi, PhD Yokohama City University
1:45pm	**Integrated Risk Assessment Methodology for Endocrine Disrupting Chemicals (IRAMe)** Kiyoung Lee, ScD, CIH Seoul National University, Korea
2:15pm	**Current Chemical Management and Prioritization in Taiwan** Steve Lin, PhD Safety and Health Technology Center (SAHTECH), Taiwan
2:30pm	**Introduction to Agency for Science, Technology, and Research (A*STAR)** Kenneth Lee, PhD Agency for Science, Technology & Research (A*STAR), Singapore
2:45pm	**Integrated Approaches to Testing and Assessment (IATA) Case Studies Project** Robert Diderich Organisation for Economic Cooperation and Development (OECD)
3:15pm	**BREAK**
3:45pm	**The Frank R. Lautenberg Chemical Safety for the 21st Century Act** Jeff Morris Office of Chemical Safety and Pollution Prevention (OCSPP), US EPA
4:15pm	**Case Example for Use of High Throughput and Computational Approaches in Decision Making for Endocrine Disruption Potential** Stanley Barone, PhD Office of Chemical Safety and Pollution Prevention (OCSPP), US EPA
4:45pm	**California's Approach to Evaluating and Incorporating New Methods in Prioritization and Risk Assessment** Gina Solomon, MD, MPH California Environmental Protection Agency (CalEPA)

5:15pm	**Closing and Considerations for Tomorrow** Robert J. Kavlock, PhD, US EPA
5:30pm	**Adjourn**
6:00 pm	**Group Dinner at Old Ebbitt Grill** (separate checks) 675 15th Street NW, Washington, DC

DAY 2 – THURSDAY, SEPTEMBER 15, 2016

9:00 am	**Review of Activities of Day 1** Robert J. Kavlock, PhD, US EPA
9:15 am	**Use of All Available Data in Accelerated Chemicals Assessment** John R. Bucher, PhD Associate Director, National Toxicology Program National Institute of Environmental Health Sciences (NIEHS)
9:45am	**Development of the RapidTox Decision Support Tool for 21st Century Chemical Risk Assessment** Russell S. Thomas, PhD Director, National Center for Computational Toxicology Office of Research and Development (ORD) US EPA
10:15am	**BREAK**
10:30am	**Facilitated Discussion: What Do Regulators Need to Accelerate Risk Assessment?** Co-Chairs: Stanley Barone, PhD, US EPA, and Maurice Whelan, PhD, European Commission JRC Presentation: Mike Rasenberg, EChA
12:15pm	**LUNCH**
1:15pm	**Facilitated Discussion: What Are the Chemicals of Common Interest Internationally?** Co-Chairs: Tala Henry, PhD, US EPA, and Kerry Nugent, PhD, NICNAS Presentation: Matthew Gagné, Health Canada
2:30pm	**Facilitated Discussion: How Do We Develop Shared Case Studies to Address the Challenges?** Co-Chairs: Tina Bahadori, ScD, US EPA, and Kenneth Lee, PhD, A*STAR
4:30 pm	**Agreement on All Action Items, Timeline, and Leads** Co-Chairs: Robert J. Kavlock, PhD, US EPA, and Jeff Morris, US EPA

5:00 pm **Summary/Close**
Robert J. Kavlock, PhD, US EPA

APPENDIX B: LIST OF PARTICIPANTS

Michael Babich
Director, Division of Toxicology & Risk Assessment
US Consumer Product Safety Commission

Tina Bahadori
National Program Director, Chemical Safety for Sustainability
US EPA

Stan Barone
Director, Office of Science Coordination and Policy
US EPA

Tara Barton-Maclaren
Manager, Healthy Environments and Consumer Safety Branch
Health Canada

John Bucher
Associate Director, National Toxicology Program
US National Toxicology Program

Thomas Burke
Deputy Assistant Administrator, Office of Research and Development
US EPA

Warren Casey
Director, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
US National Toxicology Program

Daniel Chang
Computational Chemist, Risk Assessment Division
US EPA

Peiying Chuan
Assistant Head, Food, Nutrition, and Consumer Care Cluster
A*STAR, Singapore

Vince Cogliano
Director, Integrated Risk Information System
US EPA

Kacee Deener
Senior Science Advisor, Office of Research and Development
US EPA

Robert Diderich
Head, Environment, Health and Safety Division
OECD

Jean-Lou Dorne
Senior Scientific Officer, Scientific Committee and Emerging Risks Unit
European Food Safety Authority

David Dix
U.S. EPA (on detail to NIH NCATS)

Lynn Flowers
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